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Phylogenetics and *in silico* Docking Studies Between Coat Protein of Mimosa Yellow Vein Virus and Whey α-lactalbumin

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ABSTRACT

Begomovirus associated symptoms were observed in several *Mimosa pudica* plants growing in crop fields of Lakshmangarh, Rajasthan (India). Amplification of a PCR product was found up to the expected size (~570 bp) during agarose gel electrophoresis only in infected samples not in healthy samples. The PCR product was cloned and partially sequenced (GenBank accession HQ876467) and sequence analysis of HQ876467 by using BLASTn revealed the highest 98% nucleotide sequence identities with Ageratum yellow vein china virus (AJ558120). GeneBank-NCBI generated CDC for HQ876467 from 1 to 774 (ADW83735) and it was utilized for *in silico* analysis. In Neighbor-Joining tree, the coat protein of mimosa yellow vein virus (ADW83735) place in monophyletic clusters of 74 bootstrap values with AV1 protein of Ageratum yellow vein China virus (CAD90081). The closest homologue of coat protein was 2B7R|A, with highest sequence identity only 37% that was selected as representative model using homology modeling softwares. Homology modeling study of coat protein (ADW83735) and docking between α-lactalbumin and coat protein was carried out by using modeling and docking software (Hex 6.3). The ADW83735 model was validated by using Procheck server for reliability that results shown only 49.2% of residues present in core region. On the basis of RMS and energy values, the best docking orientation -1.00 was selected. This study will be used for the screening of inhibitors against mimosa yellow vein virus proteins and can be further applied in future antiviral agent designing.

Key words: Mimosa yellow vein virus, coat protein, genbank, 2B7R|A, RMS

INTRODUCTION

Mimosa pudica (Fabaceae) is a creeping annual or perennial herb that grown for its curiosity value in tropical and sub-tropical parts of India. It is also known as sensitive plant in English because its leaves shows inward folding movement when touched or shaken and re-opens after few minutes later (Seismonastic movement). *Mimosa pudica* is an invasive species of crops and waste areas (Boa *et al*., 2009) and have ornamental and medicinal value (Nayagam and Pushparaj, 1999).

Begomovirus associated symptoms were observed in several *Mimosa pudica* plants growing in crop fields of Lakshmangarh, Rajasthan (India). The symptoms of the disease consist of shortening of leaf, leaf yellowing and stunting of plants. Begomovirus was suspected as a causative pathogen because of observed large population of whitefly (*Bemisia tabaci,* the vector of *Begomovirus* (Geminiviridae)) on the plants (Markham *et al*., 1994).

International Committee on the Taxonomy of Viruses (ICTV) recognized Geminiviruses on the basis of their unique virion morphology and possession of ssDNA as their genomic material (Fauquet *et al*., 2005). Geminiviruses members are major plant pathogens in tropical and subtropical countries (Moffat, 1999; Boulton, 2003; Mansoor *et al*., 2003), affecting higher range of crops, weed and other plants that cause disastrous impact on productivity. The family, Geminiviridae members have a circular, single-stranded DNA (ssDNA) genome, approximately 2.7-5.2 kb. Based on their genome arrangement and biological properties, geminiviruses are classified into four genera: *Mastrevirus, Curtovirus, Topocuvirus* and *Begomovirus* (Stanley *et al*., 2005). Genera *Begomovirus* contains almost 200 species (Fauquet *et al*., 2008) that are transmitted by dicotyledonous infecting whitefly (*Bemisia tabaci*), having either bipartite genomes (DNA-A and DNA-B) or monopartite genomes resembling DNA-A.

DNA-A component of *Begomovirus* encode various proteins and one of them is AV1 (Coat protein). The coat protein (27 to 31 k) of *Begomovirus* is involved in various processes during the life cycle of the virus, thus providing the shell to the virus and essential for infectivity in all single-component geminiviruses and play a role in insect vector specificity and transmissibility of the virus (Unseld *et al*., 2001).

On the basis of available literature the whey proteins fractions (α-lactalbumin, β-lactoglobulin and lactoferrin) can be used as an inhibitor of Tomato Yellow Leaf Curl Virus (TYLCV) which infects tomato plants (Abdelbacki *et al*., 2010).

The PCR product of infected leaf samples of *Mimosa* were cloned and partially sequenced (GenBank accession HQ876467) and sequence analysis of HQ876467 by using BLASTn revealed the highest 98% nucleotide sequence identities with Ageratum yellow vein china virus (AJ558120). The CDC of HQ876467 from 1 to 774 (ADW83735) was utilized *in silico* analysis.

The 3D structure of proteins is remaining more conserved during evolution than its sequence (Rost, 1999), it is the base of Homology modeling. Homology Modeling is useful in the prediction of the 3D structure and function of proteins (Bagchi *et al*., 2007). Homology modeling is a multi step processes that can be summarize in four steps, includes template identification, alignment, model building and refinement and validation with various computational tools (Kopp and Schwede, 2004).

Molecular docking tools used in structural molecular biology and structure-based drug discovery (Kartasasmita *et al*., 2010). Docking predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex and it is used to predict the binding orientation of small molecule drug or antiviral agents to their protein targets also for protein-protein docking in order to predict the affinity and activity of the small molecule (Kitchen *et al*., 2004).

In the present study, *in silico* approach was used for coat protein of Mimosa yellow vein virus (ADW83735) as a possible receptor for α-lactalbumin, on the basis of homology modeling and docking (protein-protein docking) study.

MATERIALS AND METHODS

PCR amplification, cloning, sequencing and homology search for HQ876467: *Mimosa pudica* leaf samples were used for the extraction of total DNA and Polymerase Chain Reaction (PCR) was performed using a pair of universal coat protein primer (Hallan, 1998). The PCR product of infected leaf samples of *Mimosa* were cloned and partially sequenced (GenBank accession HQ876467). Sequence analysis of HQ876467 (Mimosa yellow vein virus isolate Gaur-01 coat protein (CP) gene) was done by using BLASTn, revealed the highest 98% nucleotide sequence identities with Ageratum yellow vein china virus (AJ558120). The CDC of HQ876467 from 1 to 774 (ADW83735) was utilized *in silico* analysis.

Coat protein sequence of virus Mimosa yellow vein virus (ADW83735), in FASTA format was mined from GenBank-NCBI database (>gi|321437458|gb|ADW83735.1| coat protein (Mimosa yellow vein virus)) for homology modeling and docking study.

Phylogenetic tree construction: Phylogenetic relationship of ADW83735 was determined based on multiple sequence alignment by using the CLC Main Workbench 5.7. The CLC Main Workbench is developed for Windows, MacOSX and Linux. The software for either platform can be obtained from http://www.clcbio.com. The Neighbor Joining algorithms (Saitou and Nei, 1987) were used for construction of phylogenetic trees. Bootstrap values were computed using 100 replicates to evaluate support for the groupings. This analysis clustered each one of the isolates with the other previously sequenced isolate of the respective species (Fig. 1).

Template identification, sequence alignment, model building and validation: The sequence identity should guarantee a more accurate alignment between the target sequence and template structure. The homology modeling requires a query sequence with an unknown 3D structure and the target sequence that have known 3D structure with at least 35% similarity. Template sequence were selected by a simple search submits the target (Coat protein, ADW83735) sequence to programs BLASTp (Altschul *et al*., 1997, 2005) against the PDB (Protein Databank). On the basis of high identity, lowest e-value and low gaps the high resolution having sequence was selected as a template. 2B7R|A (Chain A, Structure of E378d mutant flavocytochrome C3, Length = 571) was find best homologous and as template for ADW83735 with the highest sequence identity of 37%, positives 52% and gaps are 4%.

The FASTA sequence of query (ADW83735) was uploaded on the 3D-Jigsawn (Protein Comparative Modeling Server) for the construction of its PDB file. UCSF Chimera was used for 3D structure generation based on the information obtained from sequence alignment and 3D-Jigsawn. The PDB file of the query sequence was further utilized for 3D model energy validation and docking studies (Heinrichs, 2008).

The UCLA-DOE Structure Evaluation server provide a visual analysis of the quality of a putative crystal structure for protein. Verify3D expects this crystal structure to be submitted in PDB format. We used PROCHECK (Laskowski *et al*., 1993) to calculate the main-chain torsion angles, i.e., the Ramachandran plot for our predicted structures. The validation for structure models obtained from the three software tools was performed by using PROCHECK (Laskowski *et al*., 1996).

The Verify 3D and Procheck outcomes displayed in the form of profile search and Ramachandran plots. In order to study the energy validation of query protein, the PDB file uploaded on the Structure Analysis and Validation Server (Bowie *et al*., 1991). PDB file of query protein was utilized for the structural model construction using offline bioinformatics softwares (Fig. 2) e.g., UCSF Chimera (Prajapat *et al*., 2011).

Docking of α-lactalbumin with the active site of catalase: The FASTA sequence of α-lactalbumin (accession ACI62509, Source: *Bos taurus*) was mined from GenBank-NCBI and by the help of 3D-Jigsawn server its PDB file was designed. Automated comparative docking was done in between α-lactalbumin (ACI62509) and coat protein (ADW83735) by using program Hex 6.3 (Ritchie *et al*., 2008). Hex is a primarily docking program to demonstrate the potential for

performing fast 3D superpositions using the SPF correlation approach. The PDB files of coat protein (ADW83735) and α-lactalbumin were uploaded as inputs into Hex for protein-protein docking. These are treated as a receptor and a ligand respectively. Regularization is a procedure for fitting a protein model with the ideal covalent geometry of residues to the atomic positions of the target PDB structure (Ritchie, 2008). Based on the energy minimization the best pose of the docked complex was selected.

RESULTS

The PCR products of extracted DNA from healthy and infected leaves of *Mimosa* were showed amplification of a product of the expected size (~570 bp) during agarose gel electrophoresis only in

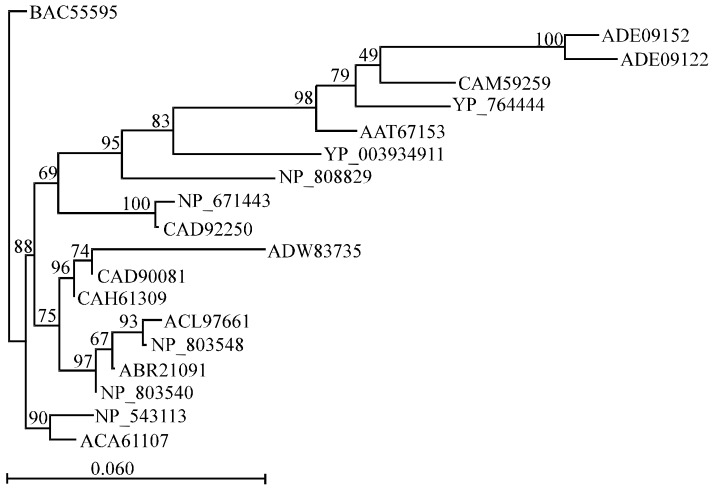

Fig. 1: A Neighbor-Joining tree based on the Coat protein [Mimosa yellow vein virus] (ADW83735), isolated from *Mimosa pudica* and other begomovirus sequences available in GenBank. Bootstrap values at major nodes are indicated. Horizontal distances are proportional to the genetic distance between isolates and vertical distances are arbitrary. Scale bar indicates the proportion of sites changing along each branch. The Coat protein [Mimosa yellow vein virus] (ADW83735) used for comparison: coat protein [Ageratum yellow vein virus-[Tomato] (BAC55595), V1 protein [Tomato leaf curl Taiwan virus] (ADE09152), V1 protein [Tomato leaf curl Taiwan virus] (ADE09122), Coat protein [Malvastrum leaf curl Guangdong virus] (CAM59259), capsid protein [Tomato leaf curl Guangdong virus] (YP_764444), Coat protein [Papaya leaf curl China virus] (AAT67153), coat protein [Tobacco leaf curl Pusa virus] (YP_003934911), Coat protein [Tomato leaf curl Malaysia virus] (NP_808829), Coat protein [Ageratum yellow vein China virus] (NP_671443), coat protein [Ageratum yellow vein China virus] (CAD92250), Coat protein [Mimosa yellow vein virus] (ADW83735), AV1 protein [Ageratum yellow vein China virus] (CAD90081), Coat protein [Ageratum yellow vein China virus] (CAH61309), AV1 [Ageratum yellow vein virus] (ACL97661), coat protein [Ageratum yellow vein Taiwan virus] 9 NP_803548), CP [Ageratum yellow vein virus] (ABR21091), Hypothetical protein [Ageratum yellow vein virus] (NP_803540), V1 coat protein [Soybean crinkle leaf virus] (NP_543113), coat protein [Ageratum yellow vein China virus] (ACA61107)

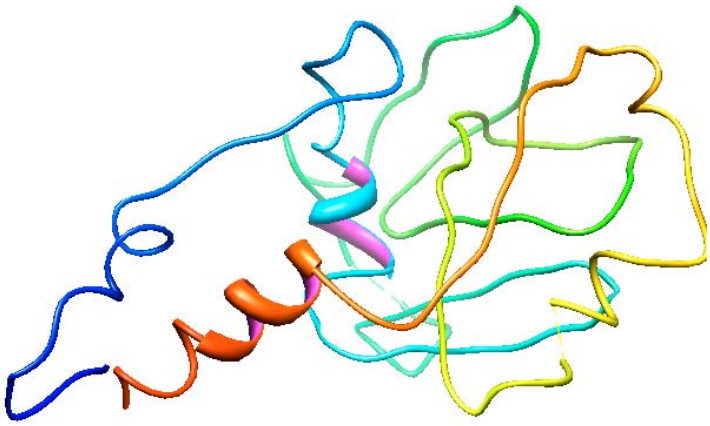

Fig. 2: Ribbon diagram of coat protein (ADW83735), designed by using UCSF Chimera

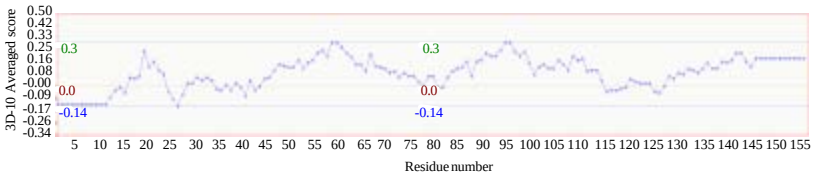

Fig. 3: The 3D profiles verified results of Coat Protein of Mimosa yellow vein virus (ADW83735), the vertical axis represents the average 3D-1D profile score for residues in a 21-residue sliding window, the center of which is at the sequence position indicated by the horizontal axis

infected samples. The PCR product was cloned, partially sequenced and submitted in NCBI (GenBank accession HQ876467). Sequence analysis by using BLASTn revealed the highest 98% nucleotide sequence identities with Ageratum yellow vein china virus (AJ558120), 97% with Ageratum yellow vein virus-[G129] (AM940137) also 97% with Ageratum yellow vein China virus-[G68] (AJ849916) and 95% with Ageratum yellow vein virus-[Tomato] DNA (AB100305). GeneBank-NCBI generated CDC for HQ876467 from 1 to 774 (ADW83735) and this was utilized for *in silico* analysis.

Phylogenetic relationship was determined based on multiple sequence alignment (Haliloglu and Bostan, 2002) of the coat protein of Mimosa yellow vein virus (ADW83735), performed by using the CLC Main Workbench 5.7. In Neighbor-Joining tree, the coat protein of Mimosa yellow vein virus (ADW83735) place in monophyletic clusters of 74 bootstrap value with AV1 protein of Ageratum yellow vein China virus (CAD90081) (Fig. 1).

The secondary structure of coat protein (ADW83735) was built by UCSF Chimera, based on the PDB file obtained from 3D-JIGSAW by using. The secondary structure of coat protein (ADW83735) has only 2 α helix and no β sheets (Fig. 2).

Verify3D works best on proteins with at least 100 residues. Verify3D analyzes the compatibility of an atomic model (3D) with its own amino acid sequence (Fig. 3).

Table 1: Results summary of the Ramachandran plot

Accession no.	Protein	Virus strain	Residues in core region	Residues in allowed region	Residues in gener region	Residues in disallowed region
ADW83735	Coat protein	Mimosa yellow vein virus	49.2%	11.4%	8.3%	2.3%

Table 2: Cluster found in Hex docking

Clst	Soln	Models	Etotal	Eshape	Eforce	Eair	Vshape	Vclash	Bmp	RMS
1	1	000:000	-582.4	-582.4	0.0	0.0	0.0	0.0	-1	-1.00

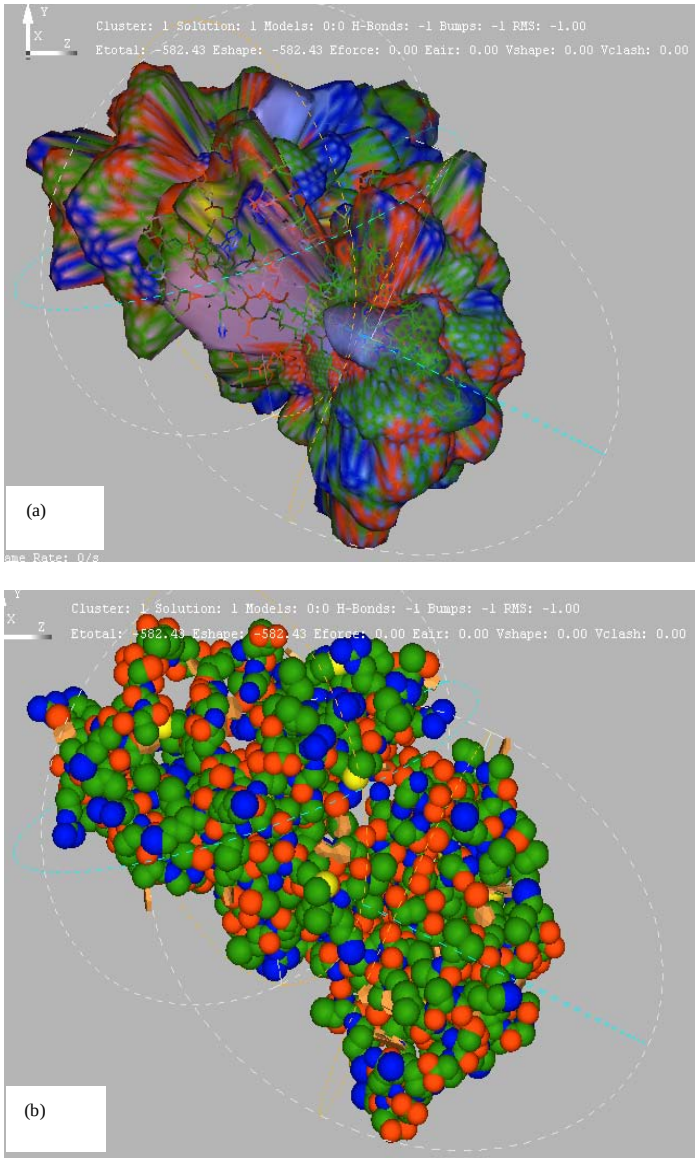

Fig. 4: The spherical harmonic surfaces for receptor coat protein (ADW83735) and ligand α-lactalbumin (ACI62509) (a) Display surface of coat protein/α-lactalbumin complex (b)

Table 3: Binding site model ADW83735 and ACI62509

Pocket/ Contouring surface for:	Polar probe	Apolar probe	Primary surface: Area	Primary surface: volume	Typical edge arc	Typical edge length (Å)	Average radius (Å)	Surface area (Å)	Triangles		
									Min	Max	Ave.
ADW83735	0.00A	0.00A	13660.73	16636.98	4.62°	1.50	18.55	9652.84	0.17	20.98	2.15
ACI62509	0.00A	0.00A	12115.81	15782.68	4.62°	1.38	17.17	8005.69	0.26	34.30	1.78

The Ramachandran plot contributes to the final values of coat protein e.g., 49.2% of residues comes in the core regions, 11.4% residues in allowed region, 8.3% residues in gener regions and 2.3 in disallowed region (Table 1).

Hex assigns multiple local coordinate systems to the larger molecule (receptor) and docks the ligand around each local coordinate frame on the receptor. Coat protein (ADW83735) has 257 long residues chain.

The binding site for coat protein model was predicted using Hex 6.3. The Etotal, Eshape and Eforce values for the model were -582.4, -582.4 and 0.0 (Table 2). On the basis of the RMS and energy values the best docking orientation was selected. The better RMS value of docking was -1.00 (Fig. 4).

In the spherical harmonic surfaces model and solid models, the coat protein (ADW83735) serve as receptor and α-lactalbumin (ACI62509) serve as ligand (Fig. 4a, b). These docking results suggest that the whey α-lactalbumin interact with the coat protein of Mimosa yellow vein virus and may block its functions (e.g., insect vector specificity and transmissibility of the virus). The binding pocket/contouring surface values for coat protein model were predicted by using Hex 6.3. The predicted contouring surface by the software with different primary surface area and volume are shown in Table 3.

DISCUSSION

The PCR primer base virus infection identification, cloning, sequencing and computational biology approaches not only speeding up the antiviral agent discovery process but also reducing the costs and provide a new dimension to structural proteomics. PCR products of extracted DNA from *Mimosa pudica* leaves, that growing in crop fields of Lakshmangarh, Rajasthan (India) were shown amplification of a product of the expected size (~570 bp) during agarose gel electrophoresis only in infected samples. The PCR product was cloned, partially sequenced nucleotide was submitted in NCBI (GenBank accession HQ876467) and it's CDC from 1 to 774 (ADW83735) was *in silico* analysis.

Sequence analysis by using BLASTn (Zhang *et al*., 2000) revealed the highest 98% nucleotide sequence identities with Ageratum yellow vein china virus (AJ558120), 97% with Ageratum yellow vein virus-[G129] (AM940137) also 97% with Ageratum yellow vein China virus-[G68] (AJ849916) and 95 % with Ageratum yellow vein virus-[Tomato] DNA (AB100305). On the basis of these results it can conclude that, it is the first report of begomovirus infection in *Mimosa pudica* in Rajastan, India.

In Neighbor-Joining tree, the coat protein of Mimosa yellow vein virus (ADW83735) place in monophyletic clusters of 74 bootstrap value with AV1 protein of Ageratum yellow vein China virus (CAD90081).

HQ876467 from 1 to 774 (ADW83735) and it was utilized for *in silico* analysis. The closest homologue of coat protein was 2B7R|A, with highest sequence identity only 37% that was selected as representative model using homology modeling softwares.

In Verify-3D graph the profile score above zero (Bowie *et al.*, 1991; Luthy *et al.*, 1992; Goh *et al.*, 2008) consider corresponds to acceptable environment of the model. ADW83735 profile score is 0.3 which indicates that environment profile of the model is good.

A good quality Ramachandran plot have over 90% in the most favoured or core regions (Prajapat *et al.*, 2010) but in Ramachandran plot of ADW83735, only 49.2% of residues present in the core regions therefore it is not a good quality model.

The interaction between the two protein molecules in 3D space can be studied by docking process and this interaction plays a significant role in structural based antiviral agent designing. The binding sites exhibit chemical specificity and the affinity that measure strength of the chemical bond (Balakrishnan *et al.*, 2010) therefore better selected RMS value for docking model was -1.00. Best docking confermation have the lowest binding energy and grater number of conformation per clustor (Babajan *et al.*, 2009). In cluster 1 of proposed model, the value of Etotal, Eshape and Eforce in the range of minus to zero.

Conserved amino acids in coat protein may form the binding cavity for the whey α-lactalbumin. Interaction of whey α-lactalbumin with coat protein, stop different function that carryout by this protein (functions e.g., insect vector specificity and transmissibility of the virus) in infected host cell and this leads to inhibit Mimosa yellow vein virus infection.

Information obtain by this study will be used in screening of other inhibitors of the begomoviral protein and can be further applied in future antiviral agent design. Homology modeling and protein-protein docking allows expanding the number of other viral protein sequences and also used to support anti-viral agent design research.

CONCLUSION

A PCR product from *Mimosa pudica* plants that shown Begomovirus associated symptoms at Lakshmangarh, Rajasthan (India), amplified up to expected size (~570 bp) during agarose gel electrophoresis only in infected samples. The PCR product was cloned and partially sequenced (GenBank accession HQ876467) and sequence analysis of HQ876467 by using BLASTn revealed the highest 98% nucleotide sequence identities with Ageratum yellow vein china virus (AJ558120). In Neighbor-Joining tree, the coat protein of Mimosa yellow vein virus (ADW83735) place in monophyletic clusters of 74 bootstrap values with AV1 protein of Ageratum yellow vein China virus (CAD90081). GeneBank-NCBI generated CDC for HQ876467 from 1 to 774 (ADW83735) and it was utilized for *in silico* analysis.

The closest homologue of ADW83735 was 2B7R|A, with highest sequence identity only 37%. Only 49.2% of residues of ADW83735 present in core region therefore this model is not fulfill the good quality Ramachandran plot criteria. On the basis of recent findings the whey proteins α-lactalbumin show antiviral activity therefore α-lactalbumin and coat protein [Mimosa yellow vein virus, ADW83735] was consider for docking study. On the basis of RMS and energy values, the best docking orientation -1.00 was selected.

This study will be used for the screening of inhibitors against Mimosa yellow vein virus coat protein and can be further applied in future antiviral agent designing because begomovirus infection cause major yield loss.

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